**Conclusion:** Besides of inducing a slight inflammatory response, mitochondrial respiratory activity dysfunction significantly potentiates the cytokine-induced inflammatory response in synoviocytes in relation with PGE2 and IL-8 release, via ROS production and NF-kB activation, contributing to chronic inflammation of synovial tissue in RA and aging joint. Resveratrol, a natural antioxidant, must be taken into account as a potential preventive or therapeutic agent in such conditions.

## 089

### PTH IMPROVES SYNOVITIS IN AN EXPERIMENTAL MODEL OF OSTEOARTHRITIS PRECEDED BY OSTEOPOROSIS

**L. Lugo**[1], M. Bellido[1], O. Sánchez-Pernaute[1], J. Roman-Blas[1], S. Castañeda[2], R. Largo[1], G. Herrero-Beaumont[1]
[1]*Fundacion Jimenez Diaz, Madrid, Spain;* [2]*Hosp. de la Princesa, Madrid, Spain*

**Purpose:** Synovitis is a major component of OA, contributing to both patient symptoms and progression of cartilage damage. Intermittent PTH administration is associated with a decrease in musculoskeletal pain in osteoporosis patients. We have already observed that PTH prevents progression of cartilage damage by improving subchondral bone quality in experimental osteoarthritis preceded by osteoporosis in rabbits. In this study, we aimed to characterize the main histopathological features of synovitis compromise in our combined animal model, as well as to determine whether intermittent administration of PTH could decrease the synovial inflammatory response.
**Methods:** OP was induced in 14 (8 month-old; 3.5-4.8 kg body weight), skeletally mature female NZ rabbits, by ovariectomy (OVX) and intramuscular injections of methylprednisolone 1 mg/kg/day for 4 weeks (OP group). Ten age and gender-matched additional animals were used as controls (Healthy group). Surgical OA was simultaneously induced in the left knees of all the rabbits (OA and OPOA groups). Twelve weeks after OVX, iPTH (1-34) was s.c. administered to 6 OP rabbits (40 μg/day, 5 days/week) for 10 wks; right knees: OP+PTH group; left knees: OPOA+PTH group). All the animals were sacrificed 22 weeks after OVX. The synovium was analyzed by Krenn grading of chronic synovitis and immunohistochemically for RAM11 and T lymphocytes. ARN was isolated to perform quantitative RT-PCR for type I collagen. Articular cartilage damage was assessed by Mankin'score. Nonpair-wise statistical comparisons were done using Mann-Whitney test for the different variables studied and the correlations were performed with Spearman test (SPSS v. 15).
**Results:** There was a positive and significant correlation between the Mankin and the Krenn scores (r=0,473, p=0,035). The synovitis score was similar for OA or OPOA groups, however it was significant reduced in OPOA+PTH vs OPOA group ($p<0.05$). Regarding the histopathology, both, OA and OPOA showed an increase of hyperplasia, synovial stroma activation and inflammation ($p<0.05$). But, there was no difference between OA and OPOA groups in any outcome. Nevertheless, PTH treatment reduced significantly hyperplasia and synovial activation. Respect to RAM11 expression, we observed a tendency to rise in OA and OPOA vs Healthy, but PTH treatment does not affect the expression of RAM11. No presence for T limphocytes was detected in any case. Type I collagen expression in synovium was increased in OA and OPOA ($p<0.05$) and reduced after PTH treatment ($p<0.05$).
**Conclusions:** In our model of OA aggravated by previous OP, synovitis correlated with OA cartilage damage. OPOA synovitis is characterized by hyperplasia and synovial stroma activation and in a lesser extent by inflammatory infiltrates. Intermittent administration of PTH succeeded in improving the synovitis, by ameliorating both hyperplasia and fibrosis.

## 090

### IF1 AND BETA SUBUNITS ARE PRESENT ON THE EXTERNAL SURFACE OF CULTURED HUMAN MENISCI AND CARTILAGE CELLS

**R. Aguilar-Gaytán**[1], C. Jiménez-Díaz[1], M. Tuena de Gómez-Puyou[2], C. Ibarra-Ponce de León[1]
[1]*Natl. Inst. of Rehabilitation, Mexico, City, Mexico;* [2]*Cellular Physiology Inst., UNAM., Mexico, City, Mexico*

**Purpose:** The $F_1$-$F_0$-ATP synthase is an enzyme complex responsible for ATP synthesis in the mitochondrial inner membrane and is driven by oxidative phosphorylation. In the last years several reports have shown that some subunits as inhibitory protein (IF1) localize in the plasma membrane of neoplasic, endothelial, tumor cells, keratinocytes and hepatic. It has been shown that α and β subunits bind angiostatin on cell surface of endothelial cells (EC) and mediate down-regulation of endothelial cell proliferation and migration. Some data suggests that angiostatin inhibits vascularization by suppression of endothelial ATP metabolism, which, in turn, may regulate vascular physiology. The angiostatin blocks synthesis and hydrolysis of ATP on the surface of endothelial cells as well as EC differentiation to form tubes and IF1 did not. IF1 does not have the anti-angiogenic effect of angiostatin, but it may attenuate the anti-angiogenic response to angiostatin. Osteoarthritis (OA) is a disorder of articular cartilage characterized by degeneration, increased cell proliferation, proteinases, growth factors, cytokines, inflammatory mediators and vascularization known as vascular pathology. At this moment, no evidence about the ectopic presence of these subunits of ATP synthase on the surface of osteoarthritic chondrocytes. Therefore we studied the dentification of the ectopic presence of IF1 and β subunits on the membrane surface of human menisci and cartilage cells.
**Methods:** The identification of the IF1 and β subunits of ATP synthase were using flow cytometry and immunofluorescence detection on cultured human menisci (vascular region) and cartilage (avascular) cells, using specific antibodies. The tissues were obtained during surgery of the patients and digested with collagenase II and cultured under 5% $CO_2$ and 37°C.
**Results:** Human menisci and cartilage cells were incubated with specific antibodies against IF1 and β subunits to show a plasma membrane location. Histograms of flow cytometry data show the increase fluorescence relative to that observed when only the secondary antibody was used. These results were corroborated by fluorescent microscopy using the same antibodies.
**Conclusions:** We showed that the surface membranes of cultured human chondrocytes and fibrochondrocytes contain the IF1 and β subunits of ATP synthase. We have to define if angiostatin is able to bind on ATP synthase and modulate angiogenesis.

## 091

### ANGIOGENESIS IN SUBCHONDRAL BONES OF OSTEOARTHRITIC KNEES

**R. Akagi**, M. Saito, N. Ikegawa, S. Yamaguchi, K. Matsuki, S. Sato, K. Nakagawa, K. Takahashi, T. Sasho
*Chiba Univ. Hosp., Chiba, Japan*

**Purpose:** Angiogenesis is implicated as a cause of many diseases as well as inevitable repair process. In osteoarthritic (OA) knees, pathological changes in the subchondral bone were considered to be related to disease initiation, progression, and a potent source of knee pain. Our previous study revealed positive immunoreactivity of Substance P, Cox-2, TNF-α, and TUJ1 in cystic lesions formed in the subchondral bone of affected compartments. These cystic lesions consist of several types of cells including CD-34 positive vascular endothelial cells. This implicates up-regulated angiogenetic activities of affected compartments. Using specimens derived at the time of total knee arthropasty (TKA) for medial-type OA knees, histological examination revealed elevated vascularity in the subcondral bone of the medial femoral condyle (MFC) compared with that of the lateral femoral condyle (LFC). In this study we investigated the relationship between angiogenetic activities of subchondral bone in OA knees and the extent of degeneration overlaying the subchondral bone.
**Methods:** Among patients who received total knee arthroplasty (TKA) in our institution, 7 patients with medial-type OA knees (one male, six female) with minimum involvement of lateral compartment confirmed with lack of bone marrow edema on Magnetic Resonance Imaging as well as macroscopic examination were selected. The mean age was 73.5 years, and every patient was classified as grade IV according to the Kellgren and Lawrence scale.Subchondral bone of the weight bearing compartment of MFC, LFC, and the anterior non-weight bearing compartment of distal femur (AF) were obtained at the time of TKA. Specimens were cultured in Dulbecco's Modified Eagle Medium with 7% Fetal Bovine Serum for 2 weeks. The culture supernatant was then added to the angiogenesis kit (KURABO, Osaka Japan) and cultured for another 11 days until vessel lumens were formed. Primary antibody against CD 31 and secondary antibody of Goat anti-mouse IgG AlkP conjugate were used to stain the vessels. The length, area, number of joints and paths of the vessels were analyzed automatically by Angiogenesis Image Analyzer (KURABO, Osaka Japan) for each compartment.
**Results:** The degrees of angiogenesis assessed by the four parameters have a tendency to be higher in the MFC compared to the LFC and the AF, but it did not reach statistical significance (Figs. 1–4).